Summary

In the past decades enzymes have become part of the chemists' toolbox and they have proven to be effective in different organic reactions. Additional to replacing traditional processes biocatalysis opened new synthetic routes not available before.

Enzymes perform their catalytic activity under mild reaction conditions with a high chemo-, regio- and stereospecificity. Therefore enzymes promise to be a green alternative for conventional processes in industry.

Polymers produced by enzymes include polysaccharides, polyesters and even vinyl polymers. The synthesis of polyamides by enzymatic pathways is however not extensively studied. This research was started to synthesize polyamides by enzyme catalysis.

Two enzymes were used to find these new synthetic routes to polyamides. *Candida antarctica* lipase B and protease papain from unripe *Carica papaya* fruit. Chapters 2-4 discuss the reactions involving the lipase and the chapters 5 and 6 discuss the use of protease papain.

Chapter 2. This chapter introduces the enzymatic ring-opening polymerization of β-propiolactam a 4-membered lactam ring. Our search for possible substrates for the *Candida antarctica* lipase B (N435) included lactams with ring sizes 4,7,9,13. From the possible substrates the 4 membered propiolactam could be polymerized at 90 °C in toluene. Drying the enzyme turned out to be critical. Optimally the N435 is dried for 24 hours over P_2O_5. When too much water is removed from, or when too much water is left in the catalyst the degree of polymerization drops. The poly(β-alanine) was characterized by MALDI-ToF MS and ^{1}H-NMR spectroscopy.

The observation that β-alanine cannot be polymerized by the N435 led to the conclusion that the reaction mechanism accepted for enzyme catalyzed polymerization of lactones does not apply in this case. In close colaboration with us a new mechanism was developed by computer modeling and it is briefly discussed.

Chapter 3. Enzymatic catalysis can also be applied to the polycondensation of diesters and diamines. In this chapter the polycondensation of diesters and diamines is followed with ATR-FTIR spectroscopy over time. A comparison is made between the rate of amide formation by the uncatalyzed reaction and by the enzymatic reaction. These measurements are repeated at different temperatures and for different monomers.

The enzymatic contribution was the highest when low temperatures are applied (7 °C and room temperature) since the uncatalyzed reaction is slow in these cases. At 60 °C the uncatalyzed reaction is faster than the enzymatic reaction. When a diester is used

with a worse leaving group (ethanol in stead of methanol) the rate of the uncatalyzed reaction drops. The enzymatic contribution is equal to the reaction with the methylester.

Three diamines were used in a polycondensation reaction with dimethyladipate, 1,4-diaminobutane, diethylenetriamine and 4,9-dioxa-1,12-dodecanediamine. From these the condensation reaction with 1,4-butanediamine was best catalyzed by N435.

Chapter 4. Shows the homopolymerization and copolymerization of amino acid esters catalyzed by the protease papain. Four hydrophobic amino acids were used, tyrosine, leucine, phenylalanine and tryptophan. After a reaction for 24 hours in buffer the products precipitate and are collected by centrifugation. A detailed analysis of the composition is done by MALDI-ToF mass spectrometry.

The reactivity of the amino acids was determined to be Tyr > Leu > Phe > Trp deducted from the DP_{avg} and yield of the polymers.

In the binary and ternary copolymerization this reactivity order was determined to be Leu > Tyr > Phe > Trp. For each copolymer the compositional distribution of all chain lengths present and DP_{avg} were calculated from MALDI-ToF spectra. When the chains grow longer in general more leucine is present and less tryptophan is incorporated in the chains. Tyrosine is slightly more reactive than phenylalanine. Phenylalanine produces short chains with apparently a low solubility.

The reactivity of the amino acids is determined by the selectivity of the enzyme and the solubility of the monomers and polymers. It is recommended that future research is directed to increasing the solubility of the products and elucidating the selectivity of papain in the synthesis of polyamino acids.

Chapter 5. Papain is able to synthesize amide bonds between protected amino acids and diamines. Chapter 5 shows that a series of monoamides based on protected glycine, phenylalanine and leucine and aromatic diamines (o-,p- and m-phenylene diamine) can be synthesized catalyzed by papain. The preferred mono amidation is explained by precipitation of the monoamide.